

*a
vite* PCR conditions. Primers used included 5'-TCCACCACCCCTGTTGCTGTAG (SEQ ID NO: 52) and 5'-GACCACAGTCCATGACATCACT (SEQ ID NO: 53).

Remarks

Applicants respectfully request entry of this amendment prior to examination of this case on the merits. The specification has been amended to insert the appropriate SEQ ID NOS corresponding to the Sequence Listing being submitted herewith. No new matter has been added by virtue of this amendment.

Conclusion

If there are any fees due in connection with the filing of this response, please charge the fees to our **Deposit Account No. 06-1448**. If a telephone conversation with Applicant's Agent would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1770.

Respectfully submitted,

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Marked-up version of replacement paragraphs of the specification showing changes made:

On page 13, the second (2nd) complete paragraph was amended as follows:

Figure 1. Primary Structure Alignments of *p53*, *p73*, and *p63*.

Human *p53*, human *p73 β* , human *Tap63 γ* are presented, with residues identical to *p53* shaded in gray, and remaining consensus residues shaded in black (SEQ ID NOs: 25, 26, 15, and 21).

On pages 13-14, the paragraph bridging pages 13-14 was amended as follows:

Figure 2. Genomic Origin and Diversity of *p63* Isotypes

(A) Schematic of human *p63* gene structure highlighting positions of exons (coding sequences in black), the two promoters in exon one (black arrow) and exon 3' (gray arrow), and the major post-transcriptional splicing events which give rise to the major *p63* isotypes.

(B) Domain structure of *p53*, *p73 α* and β , and the major *p63* isotypes, *Tap63 $\alpha\beta$* , and γ , and $\Delta Np63$ α , β , and γ , highlighting regions involved in transactivation (TA), DNA binding, and oligomerization (oligo). White box denotes 39aa N-terminal extension unique to TA**p63*. Gray box represents 14aa unique to $\Delta Np63$.

(C) Sequence alignment of N-termini of murine and human *p63* including that found in TA**p63* (SEQ ID NO: 45), TAp63 (SEQ ID NO: 46), and $\Delta Np63$ (SEQ ID NO: 47).

(D) Alignment and comparison of the human *p63* α , β , and γ C-terminal sequences (SEQ ID NOs: 48, 49, and 50).

On pages 99-100, the paragraph bridging pages 99-100 was amended as set forth below. Please note that “PCR essential data” appearing in line 21 of this paragraph was underlined in the original text.

In another embodiment, subject the kits of the invention provide compositions and reagents useful for the detection of one or more *p63* gene mRNA product. For example, exemplary compositions for inclusion in the kits include one or more *p63* mRNA primers for use in detecting or, preferably, quantitating one or more *p63* mRNA transcripts present in a sample

of animal cells or a tissue biopsy. For example, a PCR primer specific to a *p63* mRNA such as a human TAp63-encoding mRNA (e.g. the PCR primer pair 5'-ATGCCAGAGCACACAG-3' (SEQ ID NO: 51) and 5'-AGCTCATGGTTGGGCAC-3') (SEQ ID NO: 42); or a human DNp63-encoding mRNA (e.g. the PCR primer pair 5'-CAGACTCAATTAGTGAG-3' (SEQ ID NO: 43) and 5'-AGCTCATGGTTGGGCAC-3') (SEQ ID NO: 42). Other exemplary PCR primers useful for detecting a *p63*-expressing mRNA may be obtained by analysis of a *p63*-encoding gene sequence as provided herein. Optionally, preferred PCR primer pairs may be obtained by computer analysis of a subject gene sequence using computer software known to the skilled artisan (e.g. Primer Express 1.0 Software by PE Biosystems). In certain instances, the kit may include a control probe or primer to confirm the integrity of the mRNA population sampled. For example, PCR primers for use in kits of the invention include control PCR primer pairs capable of amplifying a housekeeping gene such as a glyceraldehyde-3-phosphate dehydrogenase gene (e.g. the PCR primer pair 5'-TCCACCACCCCTGTTGCTGTAG-3' (SEQ ID NO: 52) and 5'-GACCACAGTCCATGACATCACT-3') (SEQ ID NO: 53). Optionally, the kit may contain one or more reagents useful for the isolation of RNA from a cell sample or a buffer useful for mRNA hybridization analysis or PCR amplification and detection. Exemplary reagents and conditions for performing PCR may be found in PCR essential data (edited by C.R. Newton (1995) J. Wiley & Sons). In preferred embodiments, the kit provides reagents sufficient to enable the skilled artisan to perform Real-Time quantitative Polymerase Chain Reaction (RT-PCR) analysis on an animal cell sample. Methods and equipment for the quantitative analysis of mRNA expression levels by RT-PCR are known by those of skill in the art.

On page 102, the second (2nd) complete paragraph was amended as follows:

It has been observed that the intron-exon organization was conserved between p73 and p53 (Kaghad et al., 1997), and from known exon and intron sizes for these two genes, it was possible to identify new members of this gene family using a PCR-based strategy of amplifying two exons in a conserved domain and their intervening intron. Sequence similarity in exonic regions would demonstrate a related gene, while differences in size and/or sequence or introns from p53 and p73 would indicate a novel family member. Non-degenerate and degenerate primers were designed based in sequence homology among p53 and p73 cDNAs from various

species. Primers (5'-GGCCTCGAGTACAAITWCATGTGTAAYAG (SEQ ID NO: 27) and 5'GGCATCGATTCTCTCCAGGGCAAGCACA) (SEQ ID NO: 28), designed to anneal to regions in exon 7 and exon 8, respectively, of p73 and p53, were used to amplify products from human and mouse total genomic DNA with the following conditions:

pre-PCR: 80°C 2 min, add TAQ polymerase, 94°C 5 min.

'Touchdown PCR': 94°C 1 min, 65°C 1 min, 72°C 2 min for 3 cycles:

94° 1 min, 64°C 1 min, 72°C 2 min for 3 cycles; 94°C 1 min, 63°C 1 min, 72°C 2 min for 3 cycles; 94°C 1 min, 62°C 1 min, 72°C 2 min for 2 cycles; 94°C 1 min, 61°C 1 min, 72°C 2 min for 2 cycles; 94°C 1 min, 60°C 1 min, 72°C 2 min for 2 cycles; 94°C 1 min, 59°C 1 min, 72°C 2 min for 2 cycles; 94°C 1 min, 58°C 1 min, 72°C 2 min for 20 cycles; 72°C 7 min

On page 106, the second (2nd) complete paragraph was amended as follows:

5' Rapid Amplification of cDNA Ends (RACE) was used to obtain further sequence information on p63 not contained within the murine genomic clone. Total RNA was isolated from c15 embryos lacking both p53 and p73, generated from mice bearing targeted mutations in both genes, and used as the template in a first stand cDNA synthesis reaction with a murine p63-specific primer (5'-GGCATCGATGAACTCACGGCTCAGCTC) (SEQ ID NO: 29). An 'adapter' primer (5'-TTTAGTGAGGGTTAATAAGCGGCCGCGTCGTGACTGGGAGCGC) (SEQ ID NO: 30) was then ligated to the cDNA product using T4 RNA ligase. PCR was subsequently performed on the ligation product using primers (5'-GCCCTGGAGGCAGGCCGCTTATTAACCCTCAC (SEQ ID NO: 31) and 5'-GGCATCGATGTAGACAGGCATGGCACG) (SEQ ID NO: 32) with the conditions described in I. An approximately 610bp amplicon was generated, subcloned into pCDNA3 vector, and sequenced in its entirety. The 5'RACE product yielded a sequence corresponding to a N-terminal truncated form of murine p63.

On pages 106-107, the paragraph bridging pages 106-107 was amended as follows:

A bacterial plasmid cDNA library was constructed from mRNA isolated from e15 embryos lacking both p53 and p73, described above, and screened for p63 cDNAs. Hybridization screens were done essentially as described in V., using a probe, corresponding to exons 5 to 9 of p63, generated by RT-PCR on total p73-/-;p53-/- mouse RNA with primers (5'-GGGCTCGAGCTGAAGAAGCTGTACTGC (SEQ ID NO: 33) and 5'-GGGATCGATCTCCGTTCTTGATGGAA) (SEQ ID NO: 34). Three clones were identified and sequenced in their entirety. These corresponded to three different, full-length splice variants of murine p63.

On pages 113-114, the paragraph bridging pages 113-114 was amended as follows:

EMSA were performed essentially as described in Yang, A. et al., (1998), Mol Cell 2, 305-316. Briefly, human 293 kidney cells were transfected with p53, p63, and GFP expression vectors, as indicated in Figure 25, using the calcium phosphate transfection method previously described (Heald et al., 1993, Cell 74, 463-474.; Yang et al., 1998). Cells were lysed in 150 ml detergent lysis buffer (50mM Tris pH 8, 150 mM NaCl, 0.1% Triton X-100) ~24 hrs after transfection. Lysates were then incubated for 1 hr at room temperature with 100pM 32P radiolabeled, double-stranded oligonucleotides in binding buffer (16mM Hepes-KOH pH 7.5, 60 mM Kcl, 30 mM NaCl, 10% glycerol, 1mM dithiothreitol, 10 mg/ml BSA). The following oligonucleotides were used, with annealing of oligonucleotide pairs performed prior to incubation with lysate extracts above.

PG: 5'-CCTGCCTGGACTTGCCTGG + 5'-CCAGGCAAGTCCAGGCAGG (SEQ ID NOS 35 & 36, respectively, in order of appearance).

WAF: 5'-AACATGTCCCCAACATGTTG + 5'-AACATGTTGGACATGTTC (SEQ ID NOS 37 & 38, respectively, in order of appearance).

MG: 5'-CCTTAATGGACTTAATGG + 5'-CCATTAAAGTCCATTAAGG (SEQ ID NOS 39 & 40, respectively, in order of appearance).

On page 114, the second (2nd) complete paragraph was amended as follows:

Total RNA was isolated from tissues and cell lines using RNazol, dissolved in 10mM Tris pH8, 1mMEDTA (TE), and quantified using ultraviolet absorption at 260 nm. RT-PCR reactions were performed with the One-Step RT-PCR kit (Gibco-BRL), using 0.25 ug total RNA in 25 ul reactions under the following conditions: 50 °C 30 min; 94 °C 2 min; 94 °C 30 sec, 52 °C 30 sec, 72 °C 1 min for 40 cycles; 72 °C 5 min. The following primers were used: human p63 TA-specific reaction: 5' - ATGTCCCAGAGCCACACAG (SEQ ID NO: 41) and 5' - AGCTCATGGTTGGGGCAC (SEQ ID NO: 42); human p63 ΔN-specific reaction: 5' - CAGACTCAATTAGTGAG (SEQ ID NO: 43) and 5' - AGCTCATGGTTGGGGCAC (SEQ ID NO: 44).

On page 119, the second (2nd) complete paragraph was amended as follows:

RT-PCR analysis of *p63* transcript isoforms: RNAs from fresh frozen specimens of cervix, vaginal and vulvar mucosa were isolated using Trizol according to the manufacturing's direction (Life Technologies, Gaithersburg, MD) and quantified spectrophotometrically. Samples tested included normal squamous mucosa or exfoliated cells (9), atrophy (6), normal or atrophic vulvar mucosa (3), and squamous carcinoma of the vulva or cervix (6). RT-PCR reactions were performed as previously described using approximately 500 ng of total RNA and the SuperScript One-Step RT-PCR System (Life Technologies, Gaithersburg, MD) . The following primer pairs were used: human *p63* TA, 5ATGTCCCAGAGCACACAG (SEQ ID NO: 41) and 5'-AGCTCATGGTTGGGGCAC (SEQ ID NO: 42); and human *p63*Δ*N*, 5'CAGACTCAATTAGTGAG (SEQ ID NO: 43), and 5'-AGCTCATGGTTGGGGCAC (SEQ ID NO: 42). Reactions were incubated in the presence of ³²PdCTP, products resolved on 8% polyacrylamide gels and identified following autoradiography as 620 (*p63* TA) and 400 (*p63*Δ*N*) base pair products respectively. To verify RNA template integrity, amplification of glyceraldehyde-3 -phosphate dehydrogenase (GAPDH, 464 bp) was performed under similar PCR conditions. Primers used included 5'-TCCACCACCCCTGTTGCTGTAG (SEQ ID NO: 52) and 5'-GACCACAGTCATGACATCACT (SEQ ID NO: 53).